Symposium 7: Cholesterol as a Regulator of Channel and Receptor Function

1055-Symp
Role Of Lipid Raft Domains In Assembly And Regulation Of Store-operated Calcium Entry Channels
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Plasma membrane lipid raft domains (LRD) serve as platforms for the assembly of signaling complexes, including Ca^{2+} signaling proteins. Such assembly determines the specificity and rate of interaction between the proteins. Previously, we have reported that (i) TRPC1 is associated with LRD, and (ii) intact LRDs are required for SOCE in salivary gland cells. Recently, Orai1 and STIM1 have been identified as key components of TRPC1-SOC channels. While the role of Orai1 is not fully elucidated as yet, STIM1 is a Ca^{2+}-binding ER resident protein that relays the store depletion signal to the plasma membrane channels. It is now well established that STIM1 aggregates in response to store depletion and translocates to the ER-PM junctional domains which are proposed to be the sites where this protein interacts with and activates channels mediating SOCE. In our recent studies, we have assessed the mechanisms that determine targeting of STIM1 and its clustering with TRPC1. Our dat demonstrate that anchoring of STIM1 in the subplasma membrane region of the cell and activation of TRPC1-dependent SOCE are determined by LRD. These findings and others describing scaffolding of TRPC1 in LRD will be discussed. Together with our studies demonstrate that orchestration of precise and dynamic assembly of SOC channels allows compartmentalization of Ca^{2+} entry signals, which is critical for regulating specific downstream signaling events that are critical for cell function.

1056-Symp
Structural Determinants of the Regulation of Kir Channels by Cholesterol
Irena Levitan.
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A variety of ion channels are regulated by cholesterol, a major lipid component of the plasma membrane whose excess is associated with multiple pathological conditions. However, the mechanism underlying cholesterol sensitivity of ion channels is unknown. We have recently shown that an increase in membrane cholesterol strongly suppresses inwardly rectifying Kir2 channels. Here we show that cholesterol sensitivity of Kir2 channels depends on a specific region of the C-terminus of the cytosolic domain of the channel, the CD loop. Furthermore, we also show that KirBac1.1, a bacterial homologue of mammalian Kir channels, is also suppressed by the elevation membrane cholesterol when incorporated into liposomes. These findings suggest that Kir channels are directly regulated by membrane cholesterol and provide first insights into the structural determinants of the sensitivity of Kir2 channels to cholesterol introducing the critical role of the cytosolic domain in cholesterol dependent channel regulation.

1057-Symp
Cholesterol Regulation of Membrane Protein Function and Sorting By Changes in Lipid Bilayer Elastic Properties
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Cholesterol regulates the function of numerous membrane proteins. This regulation may depend on specific cholesterol-protein interactions, as well as on cholesterol-induced changes in the collective properties of the cell membrane lipid bilayer. We have studied the role of cholesterol-induced changes in the bilayer elastic properties on the function of: voltage dependent sodium channels, N-type calcium channels and $GABA_A$ receptors. For all three channel types, the effects of cholesterol, which increases lipid stiffness (measured using gramicidin channels as molecular force transducers), are opposite those induced by amphiphiles that decrease bilayer stiffness. Similar correlations exist for a number of other membrane proteins. These findings strongly suggest that cholesterol can regulate membrane protein function by altering the bilayer elastic properties.
Cholesterol, similarly, has been proposed to regulate membrane protein sorting between different cellular membranes, by altering the energetic cost of the bilayer deformation associated with a mismatch between the length of a protein trans-membrane segment and the thickness of the bilayer hydrophobic core. We have investigated the energetic feasibility of such a sorting mechanism, using the continuum theory of liquid crystal deformations. The relative importance of a cholesterol-induced increase in bilayer thickness *vs*. an increase in the bilayer elastic moduli was evaluated. We find that cholesterol-induced sorting of membrane proteins indeed should be feasible - primarily due to the increase in the bilayer elastic moduli. Subsequent experimental studies support this conclusion.

1058-Symp
Cholesterol protein interaction - the issue of specificity
Gerald Gimpl.
Johannes Gutenberg University of Mainz, Mainz, Germany.

Symposium 8: Monitoring RNAs: From Single Molecules to the Cell

1059-Symp
Gene Activity in Bacteria: Beyond First Approximations
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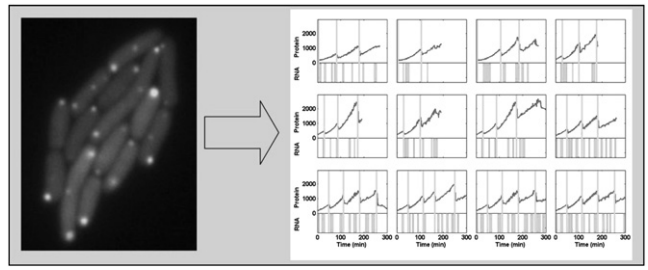
In my lab, we attempt to better understand the way a cell represents information about the environment through the activity of its genes. To achieve this aim, one has to reexamine ''first approximations'' currently used when quantifying cellular information processing: (1) the description of cellular response in term in terms of a single ''transcription rate'' rather than in terms of discrete events; (2) the treatment cellular reactions as governed by diffusion and occurring in a ''well-mixed'' cell. We use *E. coli* as a model system and study gene activity at the resolution of individual events in space and time, thus going beyond these first approximations.



1060-Symp
Using fluorescent proteins to analyze gene expression in real-time
Robert H. Singer[1], Yaron Shav-Tal[2], Xavier Darzacq[3], Valeria de Turris[1], Jeffrey Chao[1], David Gruenwald[1], Timothee Lionnet[1], Shailesh Shenoy[1].
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We developed a system using fluorescent proteins to analyze gene expression in real-time, and to follow individual mRNPs. An array of genes coding for a functional mRNA that contains 24 repeats of the MS2 coat protein binding motif combined with the MS2 coat protein fused to GFP or YFP allowed us to analyze the kinetics of transcription in real time and to detect single molecules of RNA in live cells. In these studies we used photo-bleaching of GFP-labeled mRNAs and of a YFP-polII fusion protein and photoactivation of paGFP labeled mRNA. Analysis of the complex process of transcription using fluorescent polymerase as well as fluorescent MS2 proteins provided an opportunity to model the kinetic steps of RNA synthesis. These results yield rate constants for each of the steps of promoter assembly, initiation and elongation. They demonstrate that transcription is inefficient and that polymerases can elongate faster than thought, but can pause stochastically. We have now inserted the stem-loops into the endogenous β-actin gene of mice and can follow transcription and mRNA mobility from this locus. RNAs can then be followed as single molecules in the nucleus and cytoplasm. The analysis demonstrated that subsequent RNA movements were not directed, but governed by rules of simple diffusion. The kinetics of their transport through nuclear pores yielded a biexponential kinetics. Once in the cytoplasm, mRNAs diffuse but also can be directed to their destinations by virtue of a zipcode motif in the RNA. A zipcode binding protein (ZBP1) is essential for this localization and inhibition of translation. β-actin mRNA and newly synthesized protein can thusly be localized to sites of active F-actin polymerization in migrating fibroblasts or growing neurons. Supported by NIH-EB, GM.
Key Words: single molecule imaging, RNA, regulation